Leukemogenesis: **Small differences in Myb have large effects**

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The avian retroviruses E26 and AMV carry mutated versions of the gene encoding the cellular transcription factor c-Myb. Surprisingly, these two mutant forms of Myb differ in the subsets of myeloid cells that they transform, the target genes that they activate, and the way in which they are regulated.

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What happens in leukemia when differentiation is blocked and cells proliferate in an uncontrolled fashion? This question has been extensively studied using oncogene-containing retroviruses. About a dozen of these viruses that cause specific types of acute leukemia and transform corresponding blood cells in culture have now been isolated. Isolates from viruses that contain the same oncogene have been widely assumed to transform cells by a common mechanism. E26 and avian myeloblastosis virus (AMV), two leukemia viruses that harbor closely related oncogenes derived from c-*myb*, appear to transform cells through different mechanisms, however.

The c-Myb protein is a transcription factor whose DNA-binding domain contains three homeodomain-like repeats. It is expressed in immature, proliferating cells of all hematopoietic lineages: the importance of c-*myb* in hematopoiesis is clearly demonstrated by the finding that c-Myb-deficient mice die *in utero* due to a lack of definitive blood cells. Both of the retroviral forms of Myb have truncations which delete negative-regulatory domains found in c-Myb. In addition, E26-derived Myb (E26-Myb) is fused to the Ets-1 transcription factor and AMV-derived Myb (AMV-Myb) contains a number of point mutations, three of which are located in the DNA-binding domain [1].

E26-transformed and AMV-transformed myeloid cells differ

AMV and E26 transformation results in related, but distinguishable, myeloid cells. E26 transforms cells that are myeloblast-like, can differentiate along both the granulocytic and monocytic lineages, and depend on chicken myelomonocytic growth factor (cMGF) — a cytokine related to granulocyte colony-stimulating factor (G-CSF)/interleukin (IL)-6 — for growth. In contrast, AMV transforms cells that resemble monoblasts, can be induced to differentiate into monocytes and macrophages but not granulocytes, and are cMGF independent producing their own growth factor [2]. In both cases, the v-Myb proteins do not merely block differentiation, but also impose an immature phenotype. Thus, myeloid cells transformed by a temperature-sensitive mutant of E26 can differentiate reversibly as the oncoprotein is alternately inactivated or reactivated by changing the temperature. Similarly, a regulatable form of AMV-Myb can also induce reversible changes in morphology and gene expression in myeloid cells [1,3].

Although both E26 and AMV readily transform myeloid cells in culture, only the latter can cause myeloid leukemias (E26 can induce a leukemia upon transformation of multilineage precursors, but this activity requires fusion of the Myb and Ets proteins [3]). However, a recombinant virus expressing E26-Myb together with cMGF causes an acute promyelocytic leukemia [4]. This demonstrates that autocrine growth is a prerequisite for the induction of myeloid leukemias by Myb, and raises the question of why only AMV-transformed cells produce cMGF.

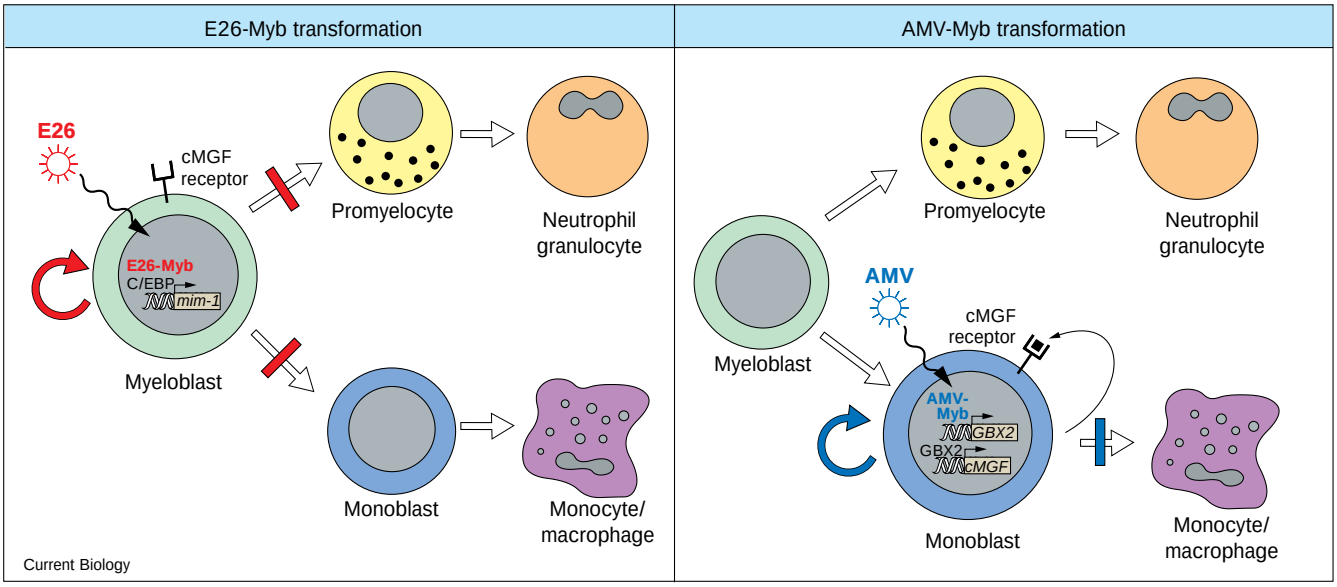
Differences in target gene activation by E26 and AMV

In spite of their similarity, the Myb proteins from AMV and E26 activate different target genes. For example, the *mim-1* gene, which encodes a secreted protein related to a chemotactic factor [5], is activated by the combinatorial action of either E26-Myb or c-Myb with the basic leucine zipper (bZip) transcription factors C/EBPα or β [1,3]. However, AMV-Myb does not induce *mim-1* expression. In contrast, AMV-Myb but not E26-Myb activates the *GBX2* gene, which encodes a homeodomain transcription factor that is expressed in AMV-transformed but not E26-transformed myeloid cells. This discovery was made by Kowenz-Leutz *et al.* [6] who, working backwards from the promoter of the cMGF gene looking for promoter-binding proteins, cloned *GBX2* as a regulator of cMGF expression and showed that the ectopic expression of *GBX2* in E26-transformed myeloblasts induces the synthesis of cMGF and the acquisition of a monoblast-like phenotype. The phenotypic and leukemogenic differences between AMV-transformed and E26-transformed cells may therefore boil down to whether or not each type of Myb protein is able to activate the expression of *GBX2*, and ultimately, the cMGF gene. This aspect of the biology of the two v-Myb proteins is summarized in Figure 1.

Mutations in AMV-Myb disrupt the binding of Cyp-40

How can two such similar versions of the Myb protein have such different biological effects? Here, three point mutations in the DNA-binding domain of AMV-Myb seem to be crucial. Thus, v-Myb constructs containing these mutations activate *GBX2* but not *mim-1* expression, whereas Myb proteins lacking these mutations (c-Myb and E26-Myb)

Figure 1



Phenotypes of cells transformed by E26-Myb and AMV-Myb in the context of myeloid cell differentiation. White arrows indicate differentiation, and red and blue arrows indicate self-renewal. Points where v-Myb proteins block differentiation are indicated by red and blue bars.

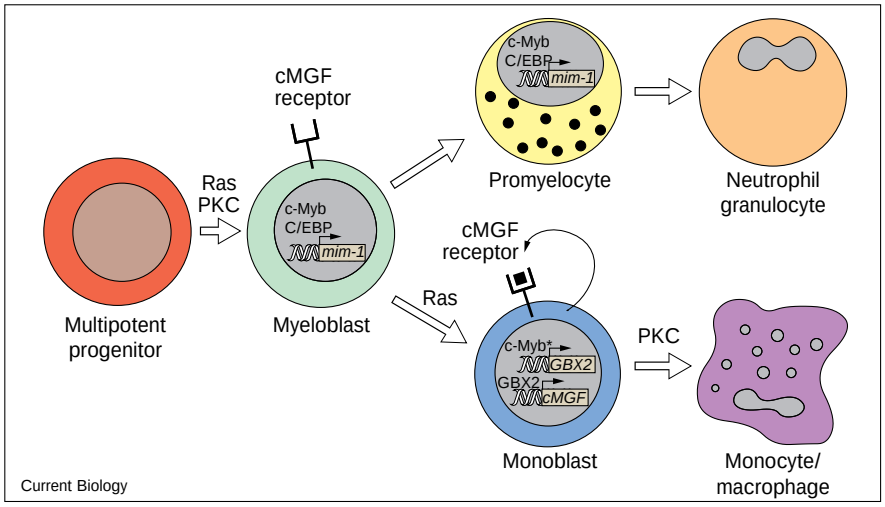
activate *mim-1* but not *GBX2* [2,6,7]. The point mutations in AMV-Myb do not affect DNA-binding capacity or specificity but, as they face away from the DNA towards the solvent, these residues probably form part of a protein interaction surface [8]. Recent work by Leverson and Ness [9] has now revealed that a cellular protein, termed Cyp-40, discriminates between the two forms of Myb. Cyp-40 is a cyclosporin-A-binding cyclophilin composed of an amino-terminal peptidyl prolyl isomerase (PPIase) domain and a carboxy-terminal domain containing tetratricopeptide repeats (TPRs). The TPR region of Cyp-40 binds to the E26-Myb/c-Myb DNA-binding domain but not to that of AMV-Myb. Furthermore, Cyp-40 inhibits the DNA-binding activity of E26-Myb/c-Myb but not that of AMV-Myb, and this inhibition can be blocked by cyclosporin A, a competitive inhibitor of PPIase activity. Although the role of Cyp-40 *in vivo* is still unclear, these observations suggest that AMV-Myb has evolved to escape negative regulation by cyclophilins. In addition, the Cyp-40 data suggest that AMV-Myb and E26-Myb proteins might interact with alternate sets of transcriptional cofactors, leading ultimately to the induction of different sets of target genes, such as *mim-1* and *GBX2*. The emerging role of cyclophilins and other PPIases as regulators of transcription, signal transduction and cell cycle is the subject of a recent review [10].

Alternate Myb activities and myeloid differentiation

The profound biological differences between E26-Myb/c-Myb and AMV-Myb suggest that the role of c-Myb in normal myeloid cell differentiation might involve two different active states of the protein. This is supported by the finding that the inability of E26-Myb/c-Myb to induce *GBX2* expression can be overcome by the activation of upstream signals. Thus, Ras or tyrosine kinase-type oncogenes or an activated epidermal growth factor receptor can each cooperate with E26-Myb/c-Myb to activate *GBX2* expression [6]. This suggests that a post-translational modification enables E26-Myb/c-Myb to interact with a cellular cofactor and that the mutations in AMV-Myb allow it to bind to this cofactor constitutively. Therefore, receptor tyrosine kinases in normal cells might ultimately control the preference of c-Myb for one set of target genes or the other.

How do these results fit into the regulation of normal hematopoiesis? The model in Figure 2 proposes that the transition of a multipotent progenitor into a myeloblast requires the upregulation of c-Myb and C/EBP(α or β), as well as the concomitant downregulation of stem-cell-specific transcription factors. This idea is based on the observation that E26-transformed multipotent progenitors upregulate C/EBP and downregulate GATA proteins when they are induced to differentiate into myeloid cells or eosinophils [11,12]. Consistent with this proposal, C/EBPα-deficient mice have a block to the development of neutrophil and eosinophil granulocytes [13]. The decision of bipotent progenitors to differentiate into granulocytes might represent a default pathway in which *mim-1* expression is maintained, although the activation of other factors, such as retinoic acid receptors, might still be required. In contrast, the decision of myeloid progenitors

Figure 2



A model for the role of c-Myb and upstream signaling pathways in normal granulocyte and macrophage differentiation. The asterisk indicates proposed protein modifications.

to differentiate towards the monocytic lineage requires the activation of a tyrosine kinase-type receptor and/or the Ras pathway, resulting in a form of c-Myb that can activate *GBX2*. In turn, monoblasts mature into monocytes and macrophages when the protein kinase C (PKC) pathway becomes activated, such as after exposure to bacterial lipopolysaccharides or phorbol esters.

The model is speculative, as neither the factor that activates the Ras/PKC pathways in multipotent progenitors to induce myeloblast differentiation, nor the factor that triggers the Ras pathway in myeloblasts to induce monoblast differentiation are known. Also, the model does not take into account several other transcription factors that have been implicated in the regulation of myeloid-specific genes such as PU.1, which has been shown to synergize with C/EBP in the regulation of these genes [13].

Any commonalities left?

The observations discussed suggest that Myb proteins might have a role as 'biochemical switches', interpreting upstream signals by activating alternate sets of genes. The mechanism by which either type of v-Myb transforms cells remains unclear, however. It has been found that both E26-Myb and c-Myb can upregulate the *bcl-2* gene, preventing myeloid cells and T cells from undergoing programmed cell death [14,15]. Although this might be an essential step for cell transformation by E26-Myb, it is not clear whether this also holds true for AMV-Myb. In addition, perhaps the most important question that remains to be answered is: how do Myb proteins prevent myeloid cells from entering cell cycle withdrawal? Will the two nonidentical twins agree for once to operate through a common mechanism? My bet is no.

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